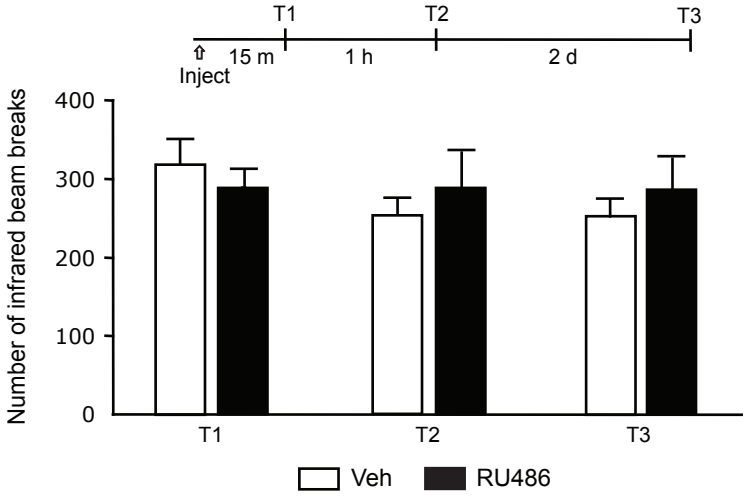
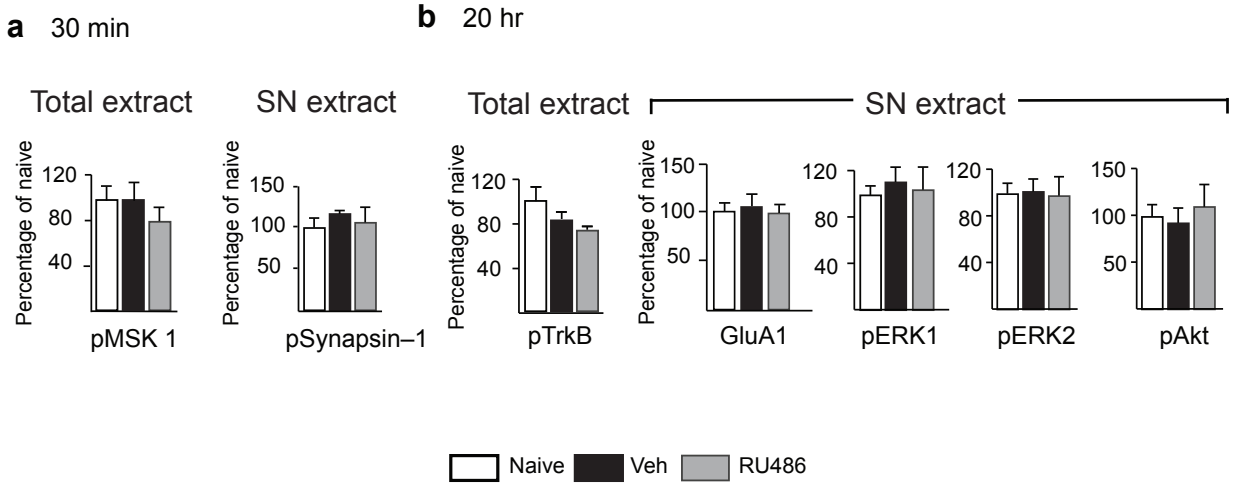
Glucocorticoid receptors recruit the CaMKIIα, BDNF–CREB pathways to mediate memory consolidation

Dillon Y. Chen, Dhananjay Bambah–Mukku, Gabriella Pollonini and Cristina M. Alberini



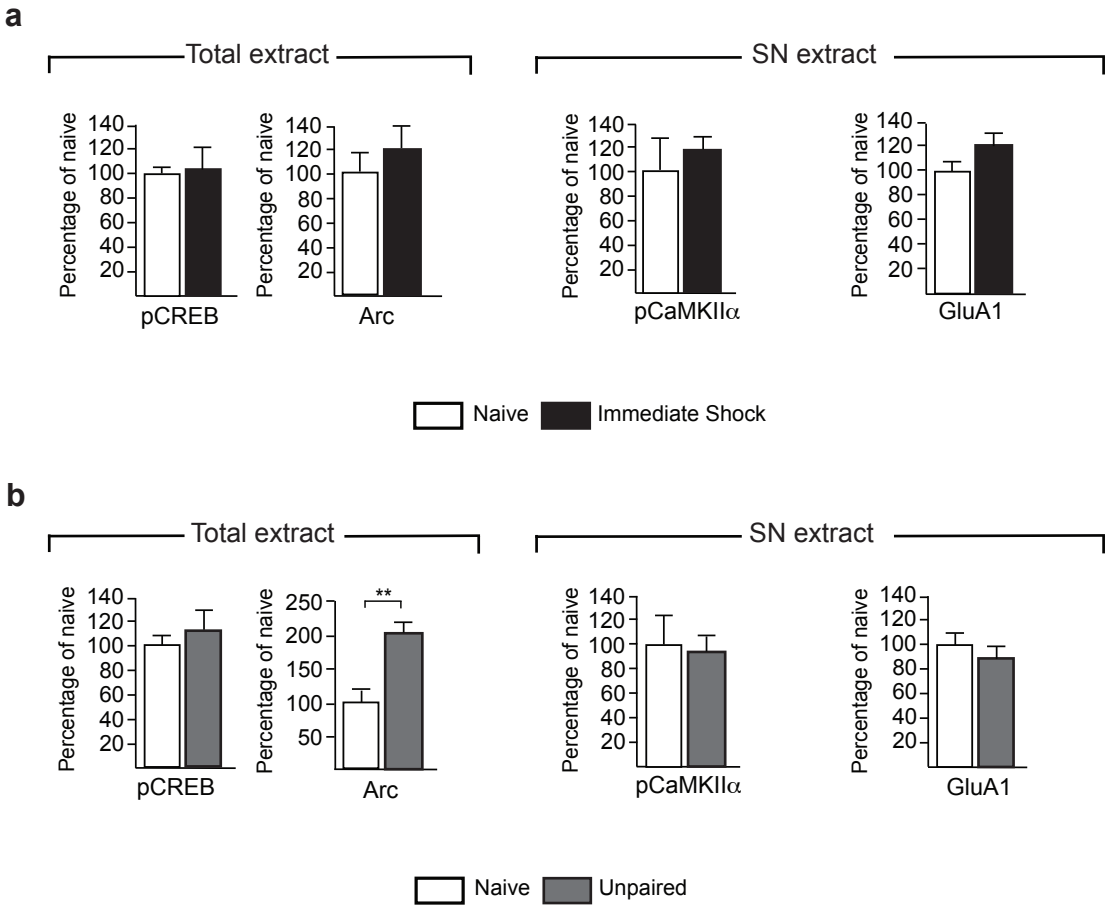
Supplementary Figure 1: RU486 does not affect spontaneous locomotor activity. Bilateral hippocampal injection of RU486 does not affect locomotor activity 15 minutes (T1), 75 minutes (T2) and 2 days (T3) after the injection. Locomotor activity was measured by counting the number of infrared beams broken within a 540 second test period in the IA chamber.T1: Veh (318.6 ± 27.6), RU486 (286.3 ± 22.6); T2: Veh (253.3 ± 20.7), RU486 (290.5 ± 40.4), T3: Veh (250.2 ± 20.4), RU486 (283.8 ± 38.4). n = 6 rats/group. T = Test. Data are expressed as mean number of infrared beam breaks ± s.e.m.

Chen et al. Supplementary Fig. 1

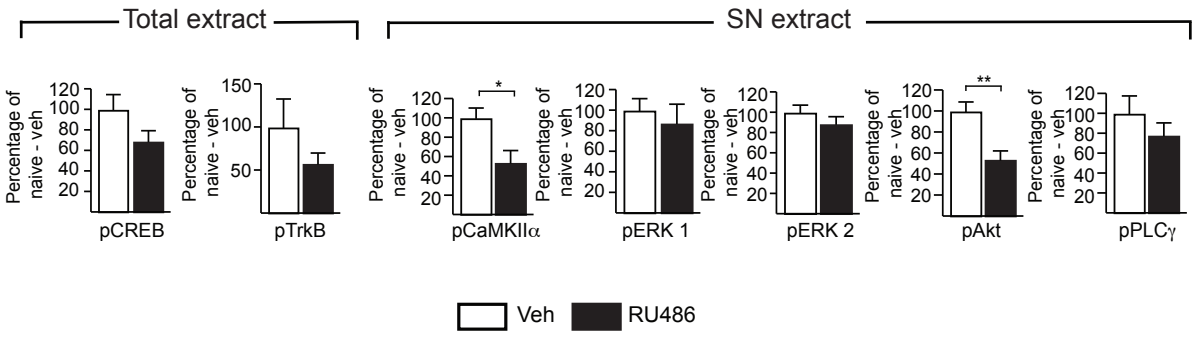


Supplementary Figure 2: Markers not significantly changed following IA training and RU486 treatment. a-b) Quantitative densitometric western blot analyses of dorsal hippocampal extracts from naive or trained rats that were bilaterally injected with either Vehicle or RU486 into the hippocampus. Neither training nor RU486 affect the levels of pMSK1 and pSynapsin–1, 30 minutes after training (**a**), or the levels of pTrkB, GluA1, pERK1/2 or pAkt, 20 hours after training (**b**) Actin was used as a loading control. Data are represented as mean percentage of naive ± s.e.m. n = 6-11 rats/group SN = synaptoneurosome. Naive = rats taken from their homecages and injected with vehicle and euthanized either 45 minutes (**a**) or 20 hours (**b**) after injection. Veh = trained rats injected with vehicle solution 15 minutes before training and euthanized either 30 minutes (**a**) or 20 hours (**b**) after training. RU486 = trained rats injected with RU486 15 minutes before training and euthanized either 30 minutes (**a**) or 20 hours (**b**) after training.

Chen et al. Supplementary Fig. 2

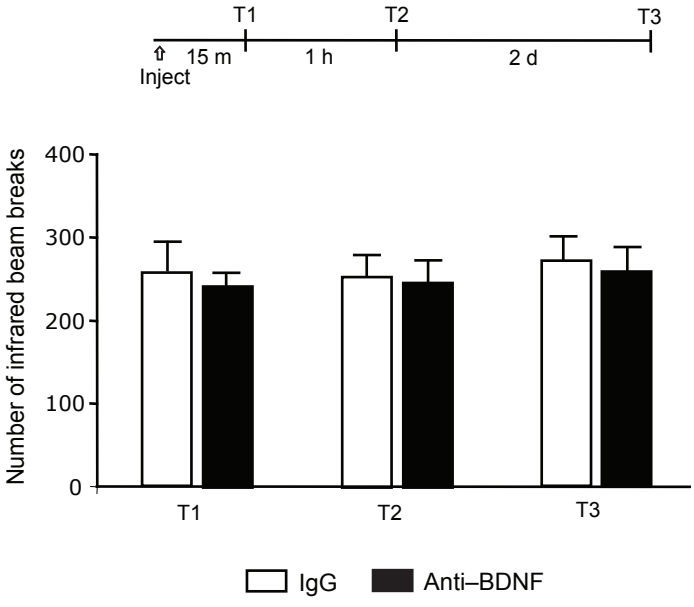


Supplementary Figure 3: Hippocampal molecular changes after immediate shock or unpaired context-shock protocol. Quantitative western blot analyses of dorsal hippocampal extracts from rats that were euthanized 30 minutes after being exposed to an immediate shock on the floor of the IA box **(a)**, or 30 minutes after being exposed to the unpaired protocol (see below) **(b)**. (**a**) No significant changes were found in the levels of pCREB (Naive: 100 ± 2.49%; Imm-Shock.: 103.58 ± 16.26%) and Arc (Naive: 100 ± 14.62%; Imm-Shock: 122.44 ± 15.23%) in the total extracts or pCamKIIα (Naive: 100 ± 25.50%; Imm-Shock: 118.91 ± 9.45%) and GluA1 (Naive: 100 ± 6.10%; Imm-Shock:122.19 ± 8.53%) in the synaptoneurosome extracts. (**b**) A significant induction of Arc (Naive: 100 ± 17.78%; Unp.: 201.12 ± 14.59%) was observed in total dorsal hippocampal extracts in the unpaired shock group. No significant changes were found in the levels of pCREB (Naive: 100 ± 7.22%; Unp.: 112.37 ± 14.92%) in the total extracts or pCamKIIα (Naive: 100 ± 23.01%; Unp.: 95.79 ± 12.37%) and GluA1 (Naive: 100 ± 8.84%; Unp.: 89.36 ± 9.17%) in the synaptoneurosome extracts. Actin was used as a loading control. Data are represented as mean percentage of naive ± s.e.m. SN = synaptoneurosome. Naive = Rats kept in the homecage. Immediate Shock (Imm. Shock) = Rats were placed directly onto the grid floor of the dark chamber of the IA box and received a shock of the same intensity (0.9 mA) as that used in IA training and were immediately returned to the homecage followed by euthanasia 30 minutes later. Unpaired (Unp.) = Rats given an exposure to the IA context in the same way as the trained rats but not shocked in the dark chamber. They returned to their home cage and, one hour later, were placed directly onto the grid floor of the dark chamber, shocked (0.9 mA), returned to their home cage and euthanized 30 minutes later. Student's *t*-test, n = 5-6 rats/group. ***P* = 0.0013.



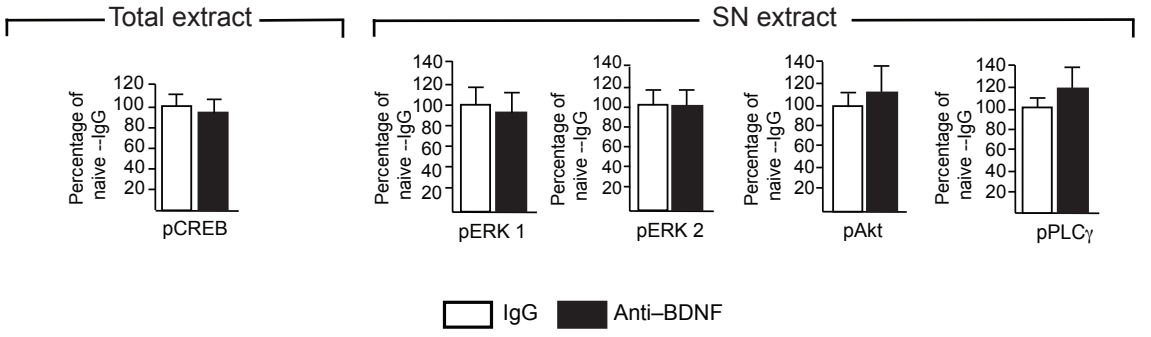
Supplementary Figure 4: Hippocampal molecular changes produced by hippocampal RU486 injection in naive rats. Quantitative western blot analyses of naive rats that were bilaterally injected with either Vehicle (Veh) or RU486 (RU) into the hippocampus show that RU486 significantly decreased the levels of pCaMKIIα (Veh: 100 ± 11.2%; RU486: 53.79 ± 13.46%) and pAkt (Veh: 100 ± 9.79%; RU486: 53.97 ± 9.15%) in the synaptoneurosomal preparation 45 minutes after the injection and resulted in non-statistically significant trends toward a decrease in the levels of pCREB (Veh: 100 ± 15.48%; RU486: 69.03 ± 11.19%,) and pTrkB (Veh: 100 ± 33.85%; RU486: 57.70 ± 13.41%) in the total extracts, as well as pERK1 (Veh: 100 ± 12.36%; RU486: 87.43 ± 19.53%) and pERK2 (Veh: 100 ± 8.13%; RU486: 88.63 ± 8.04%), and pPLCγ (Veh: 100% ± 17.33; RU486: 76.2 ± 13.33%) in the SN extracts. Actin was used as a loading control. Data are represented as mean percentage of naive ± s.e.m. SN = synaptoneurosome. Veh = naive rats taken from their homecage and injected with vehicle and euthanized 45 minutes after injection. RU486 = naive rats taken from their homecage injected with RU486 and euthanized 45 minutes after injection. Student's *t*-test, n = 6 rats/group, **P*= 0.025; ** *P* = 0.0064.

Chen et al. Supplementary Fig. 4



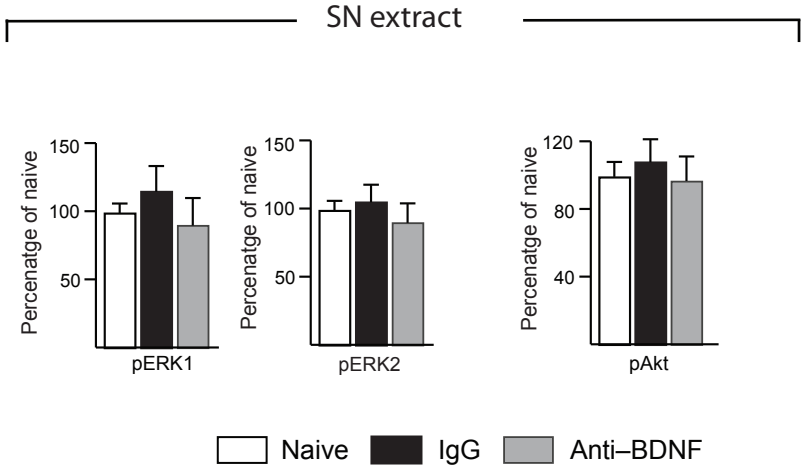
Supplementary Figure 5: Anti–BDNF does not affect spontaneous locomotor activity.
Bilateral hippocampal injection of anti–BDNF antibody does not affect locomotor activity 15 minutes (T1), 75 minutes (T2) or 2 days (T3) after the injection compared to control IgG injection. Locomotor activity was measured by counting the number of infrared beams broken within a 540 second test period in the IA chamber. T1: IgG (258.4 ± 34.5), anti–BDNF (240.7 ± 14.5); T2: IgG (253.6 ± 24.2); anti–BDNF (244.7 ± 26.3); T3: IgG (273.2 ± 26.5), anti–BDNF (260.8 ± 25.6).n = 5-6 rats/group. T = Test. Data are expressed as mean number of infrared beam breaks ± s.e.m.

Chen et al. Supplementary Fig. 5



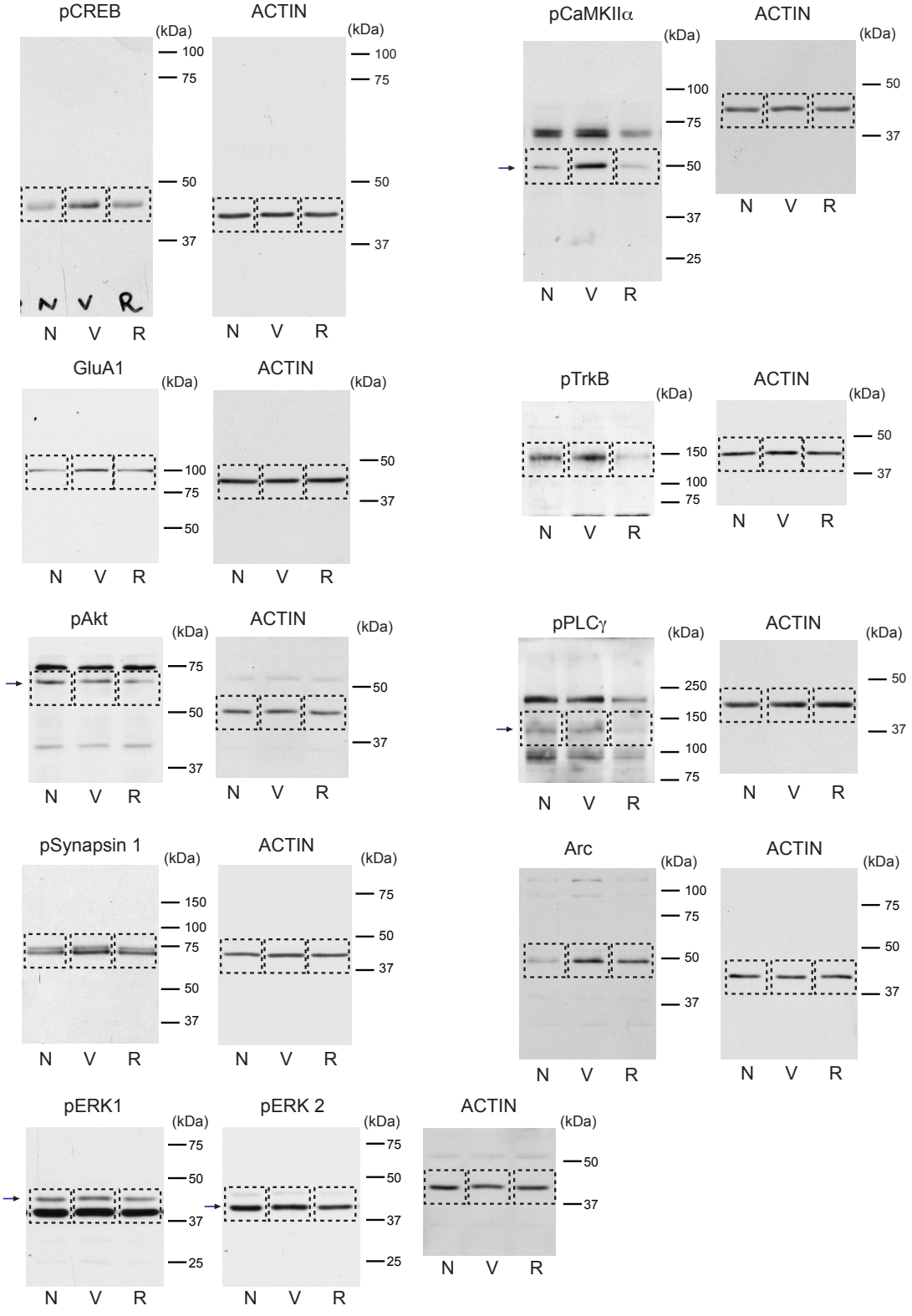
Supplementary Figure 6: Hippocampal molecular changes produced by hippocampal anti–BDNF injection in naive rats. Quantitative western blot analyses of naive rats that were bilaterally injected with either IgG or anti–BDNF antibody into the hippocampus show that anti-BDNF does not significantly alter the levels of pCREB (IgG: 100 ± 9.57%; anti–BDNF: 93.90 ± 11.29%,) in the total extracts or pERK1 (IgG: 100 ± 16.18%; anti–BDNF: 91.89 ± 15.87%), pERK2 (IgG: 100 ± 13.33%; anti–BDNF: 100.32 ± 13.46%), pPLCγ (IgG: 100 ± 7.16%; anti–BDNF: 118.09 ± 17.15%) and pAkt (IgG: 100 ± 12.25%; anti–BDNF: 113.48 ± 23.96%) in the synaptoneurosomal extracts 45 minutes after the injection. Actin was used as a loading control. Data are represented as mean percentage of naive-IgG ± s.e.m. SN = synaptoneurosome. IgG = naive rats taken from their homecage and injected with control IgG antibody and euthanized 45 minutes after injection. anti–BDNF = naive rats taken from their home cages, injected with anti-BDNF antibody and euthanized 45 minutes after injection. Student's *t*-test, n = 6 rats/group.

Chen et al. Supplementary Fig. 6



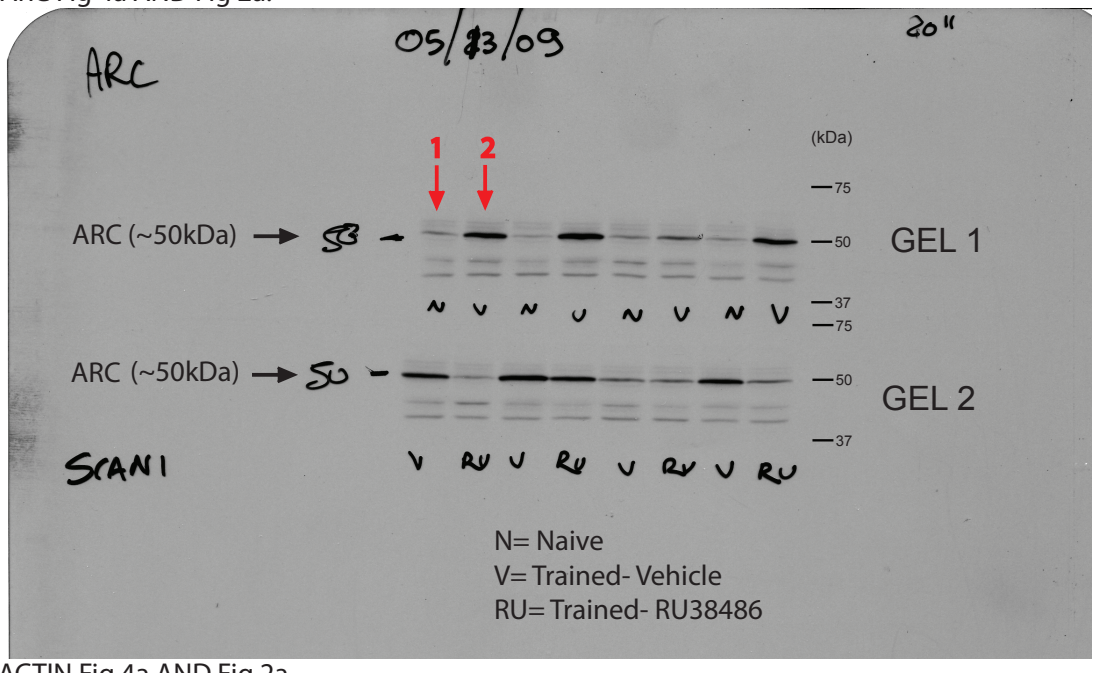
Supplementary Figure 7: Phospho- proteins that are not significantly changed by training or anti–BDNF treatment. Quantitative western blot analyses of naive and trained rats that were bilaterally injected with either IgG or anti–BDNF antibody into the hippocampus show that neither training nor anti–BDNF antibody affect the levels of pERK1/2 or pAkt 20 hours after training. Actin was used as a loading control. Data are represented as mean percentage of naive ± s.e.m. n = 5-7 rats/group. SN = synaptoneurosome. Naive = homecaged rats injected with IgG and euthanized 20 hours later. IgG = trained rats injected with IgG 15 minutes before training and euthanized 20 hours after training. Anti–BDNF = trained rats injected with anti–BDNF antibody 15 minutes before training and euthanized 20 hours after training.

Chen et al. Supplementary Fig. 7

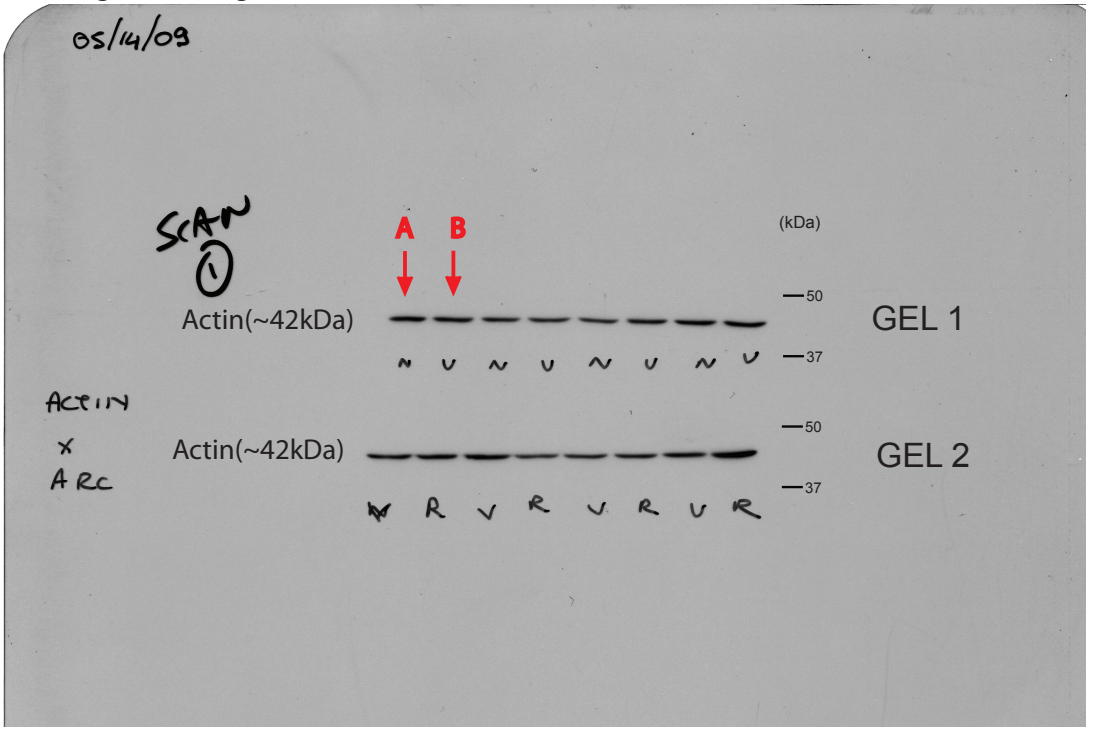


Supplementary Fig. 8: Full–length western blots of the representative images shown in Fig.2 The same memrane was first probed with the indicated antibody, stripped and then reprobed with an anti-actin antibody.

ARC Fig 4a AND Fig 2a.

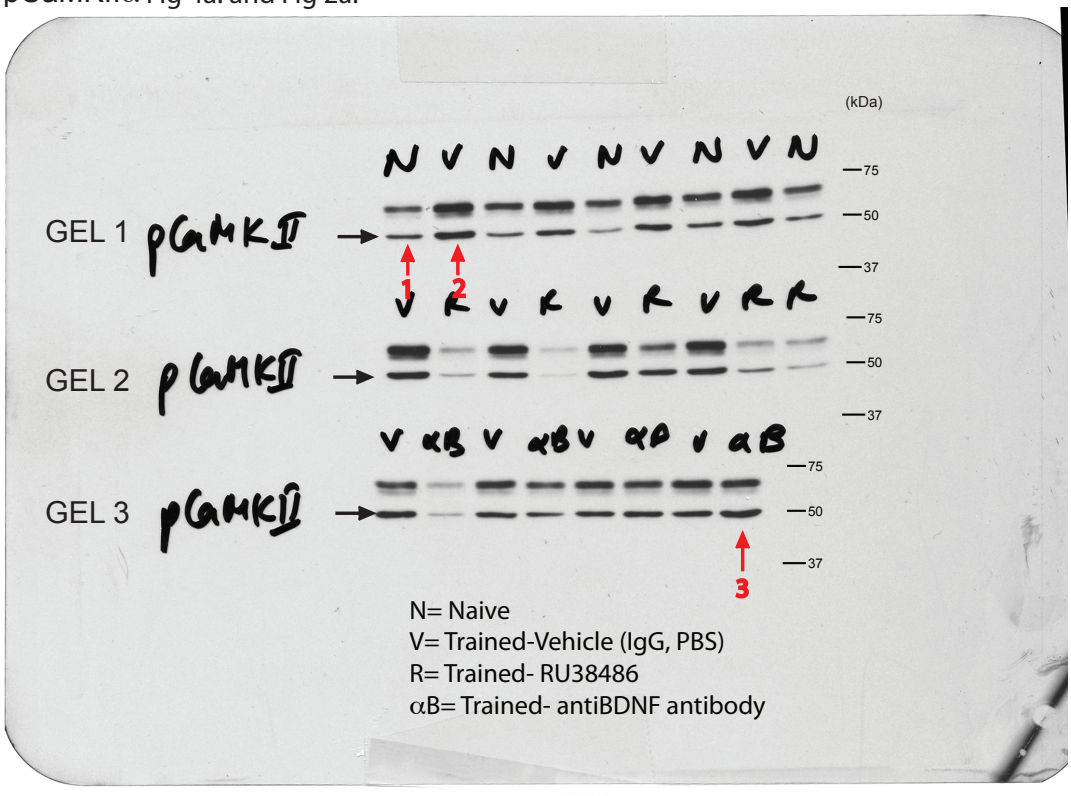


ACTIN Fig 4a AND Fig 2a.

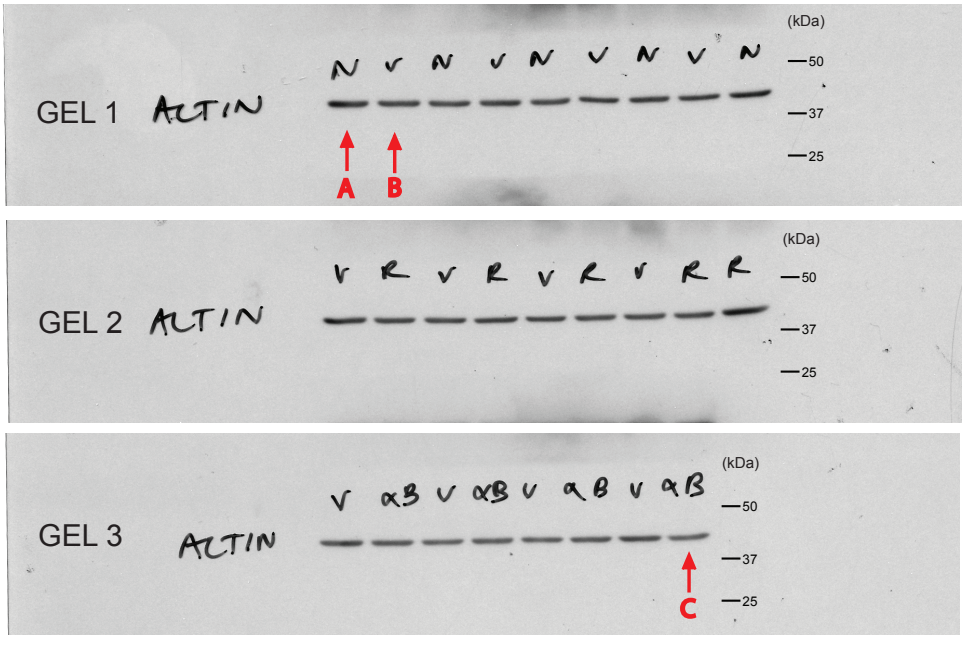


Supplementary Figure 9.: Examples of full–length western blots of the Arc data shown in Fig. 2a (Gel 2) and Fig. 4a (Gel 1). Red arrows indicate the bands that were cropped for the representative images shown in Fig. 4a (Arc). Arrows 1 and 2 indicate the Arc bands corresponding to the Naive and Trained- Vehicle groups, respectively. Arrows A and B indicate the corresponding actin bands. These are the same membranes first probed with the anti-Arc antibody, stripped and then reprobed with an anti-actin antibody.

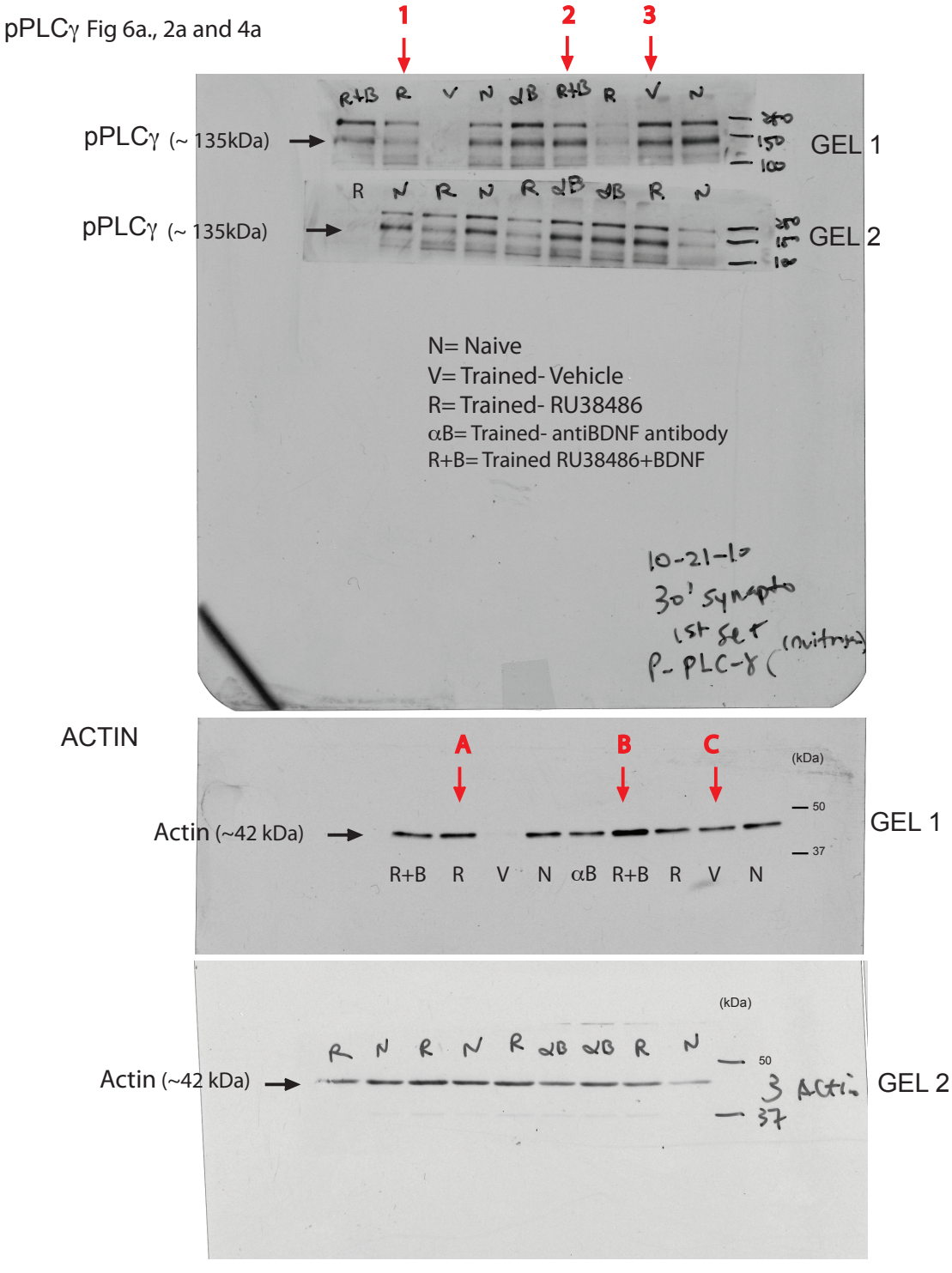
pCaMKIIα Fig 4a. and Fig 2a.



ACTIN

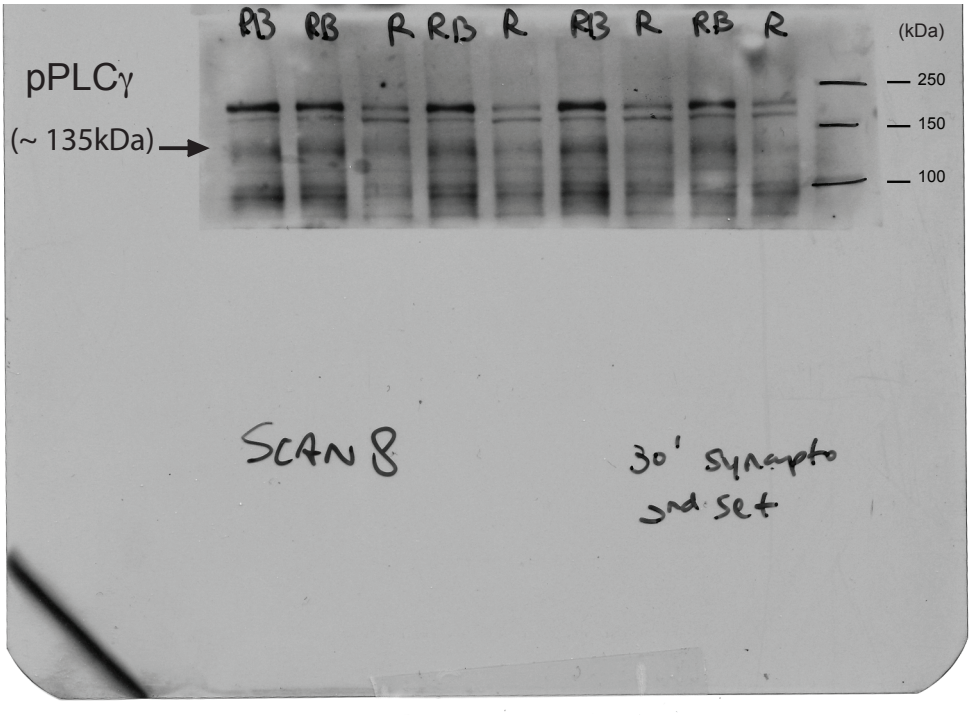


Supplementary Figure 10: **Examples of full–length western blots of the pCamKIIα data shown in Fig. 2a (Gel 2) and Fig. 4a (Gels 1 and 3).** Red arrows indicate the bands that were cropped for the representative images shown in Fig. 4a (pCamKIIα). Arrows 1, 2 and 3 correspond to the Naive, Trained- Vehicle and Trained-anti–BDNF groups, respectively. Arrows A, B and C indicate the corresponding actin bands (Same membranes reprobed).

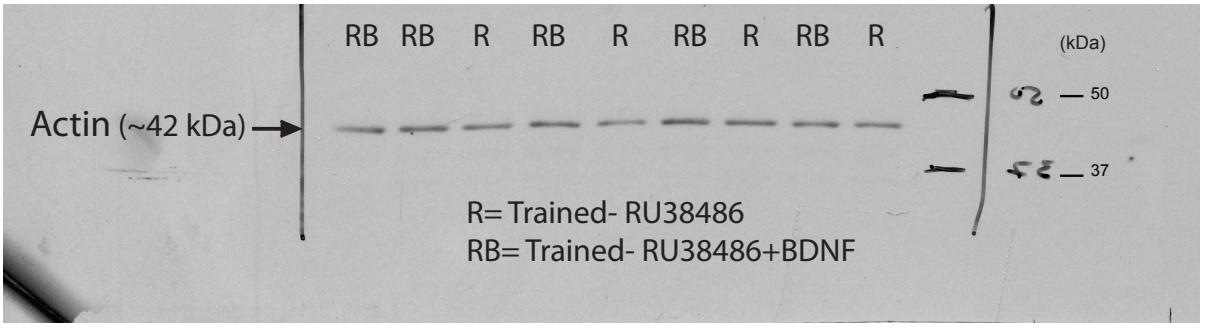


Supplementary Figure 11: **Examples of full–length western blots of the pPLCγ data shown in Fig. 6a (Gel 1), Fig. 2a (Gel 2) and Fig. 4a (Gel 2).** Red arrows indicate the bands that were cropped for the representative images shown in Fig. 6a (pPLCγ). Arrows 1, 2 AND 3 indicate the pPLCγ bands corresponding to the Trained- RU486, Trained-RU486+BDNF and Trained-Vehicle groups, respectively. Arrows A, B and C indicate the corresponding actin bands. The membranes were cut and probed in parallel for pPLCγ and actin.

pPLCγ Fig 4a.



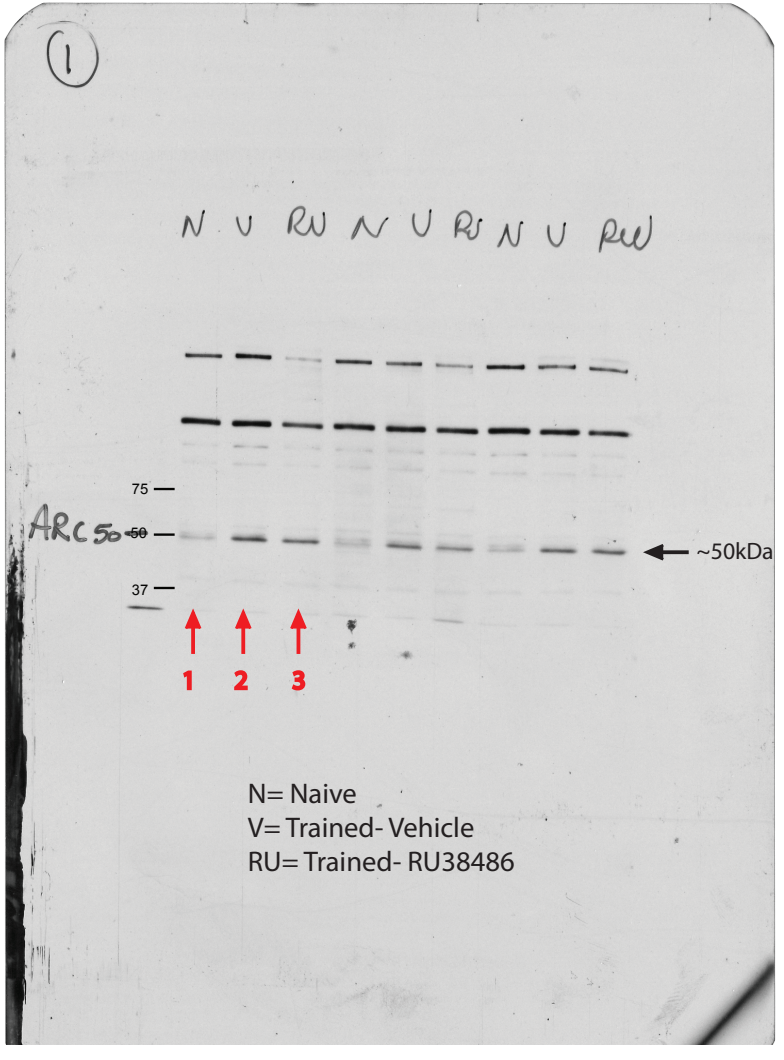
ACTIN



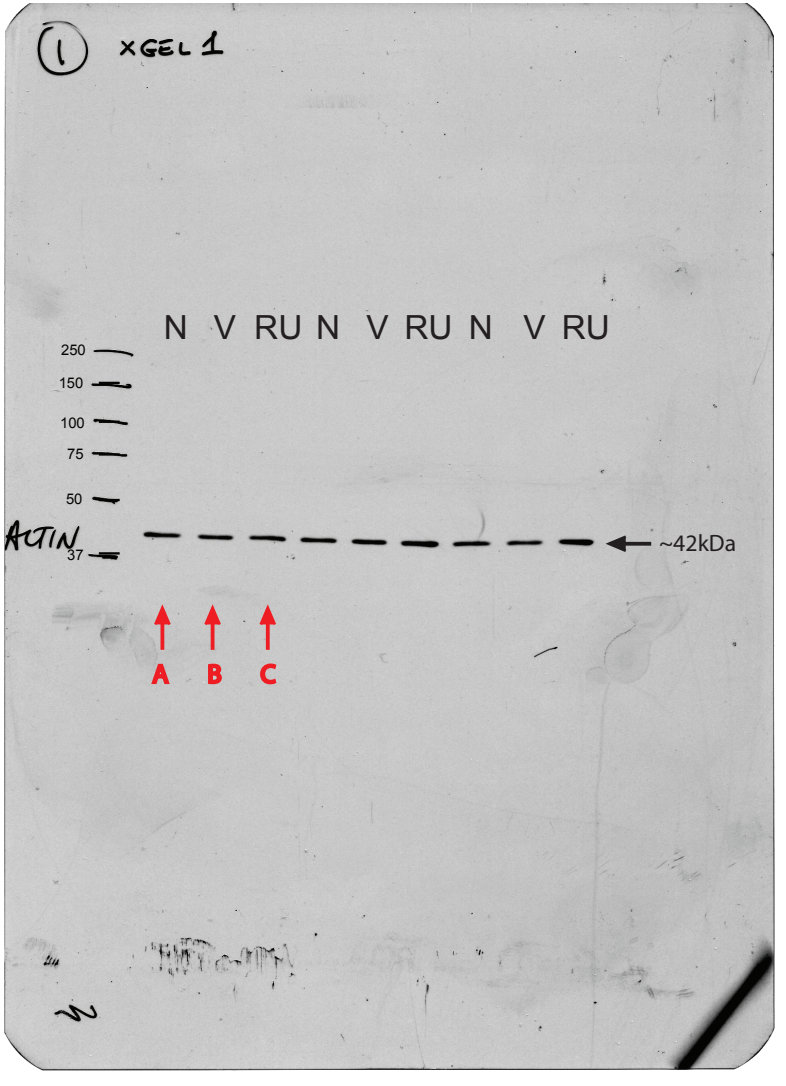
Supplementary Figure 12: Full–length western blot example for the quantitative pPLCγ results shown in Fig. 4a. The membrane was cut and probed in parallel with an anti-pPLCγ antibody and an anti-actin antibody.

Chen et al. Supplementary Fig. 12

ARC Fig.2

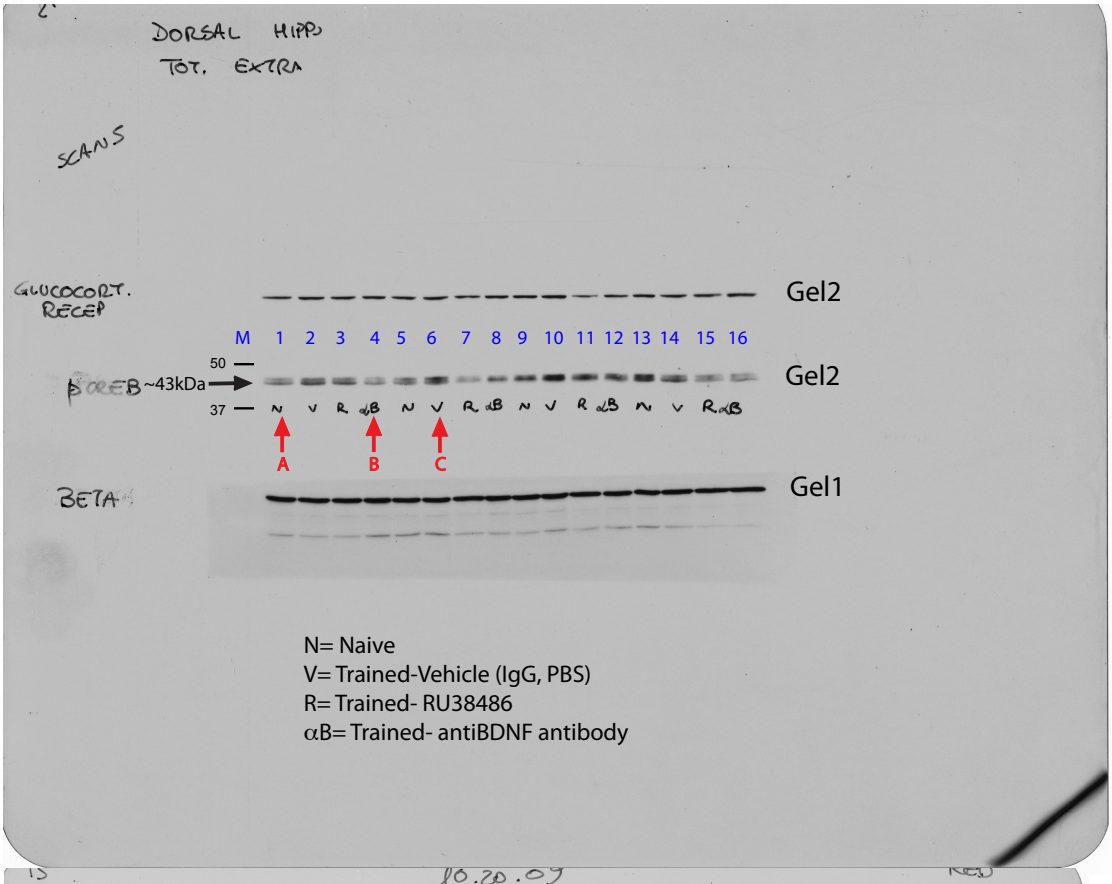


ACTIN Fig.2

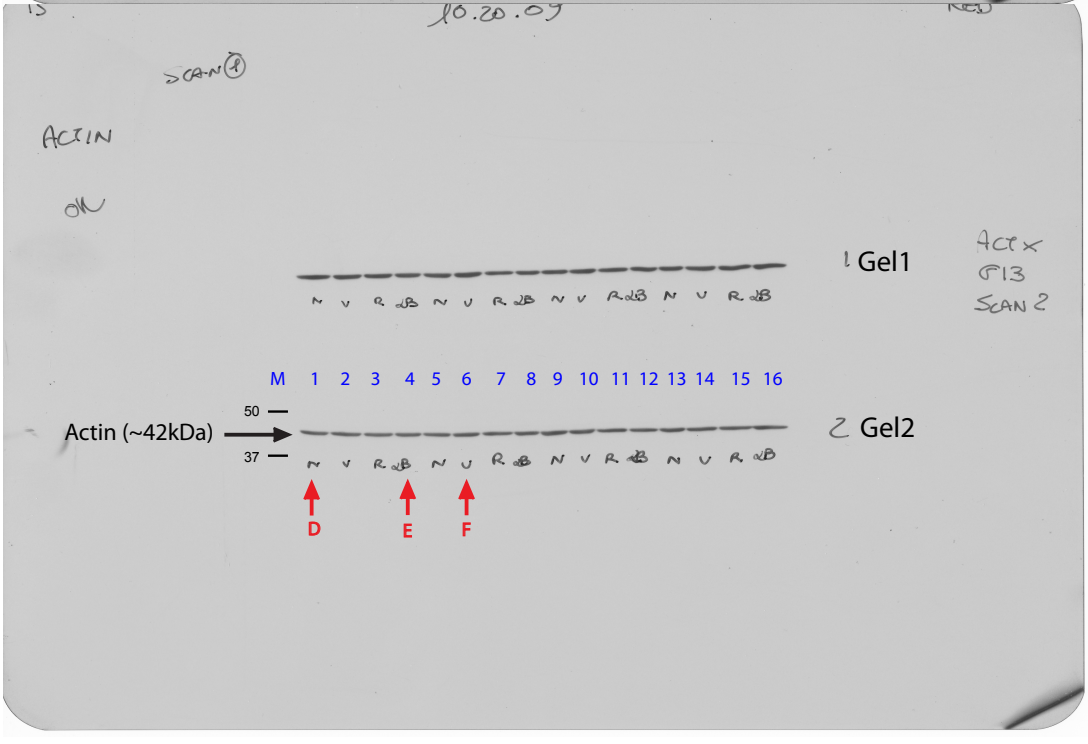


Supplementary Figure 13: Examples of full–length western blots of the Arc data shown in Fig. 2a. Red arrows indicate the samples that were cropped for the representative images shown in Fig. 2a (Arc). Specifically, Arrows 1, 2 and 3 indicate the bands corresponding to the Naive, Trained-vehicle and Trained-RU486 groups, respectively. Arrows A, B and C indicate corresponding actin bands (Same membrane reprobed).

pCREB
Fig 4b.
AND
Fig 2b.

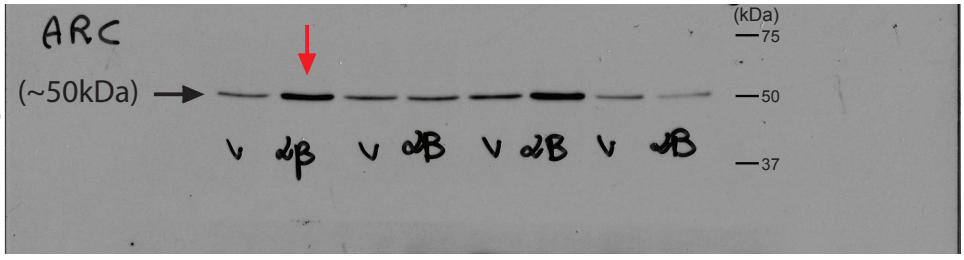


Actin
Fig 4b.
AND
Fig 2b.

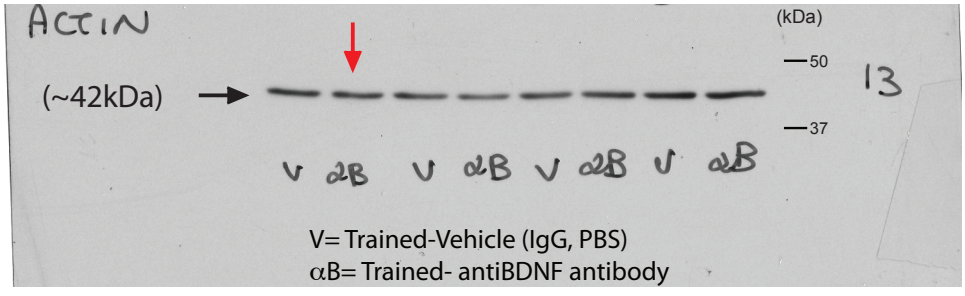


Supplementary Figure 14: Examples of full–length pCREB western blots of the data shown in Fig. 2b (Gel 2) and Fig. 4b (Gel 2). Red arrows indicate the samples that were cropped for the representative images shown in Fig. 4b (pCREB). Specifically, arrows A, B and C indicate the pCREB bands corresponding to Naive, Trained-AntiBDNF and Trained-Vehicle groups, respectively. Arrows D, E and F indicate the corresponding actin bands (Same membrane reprobed).

ARC Fig.4



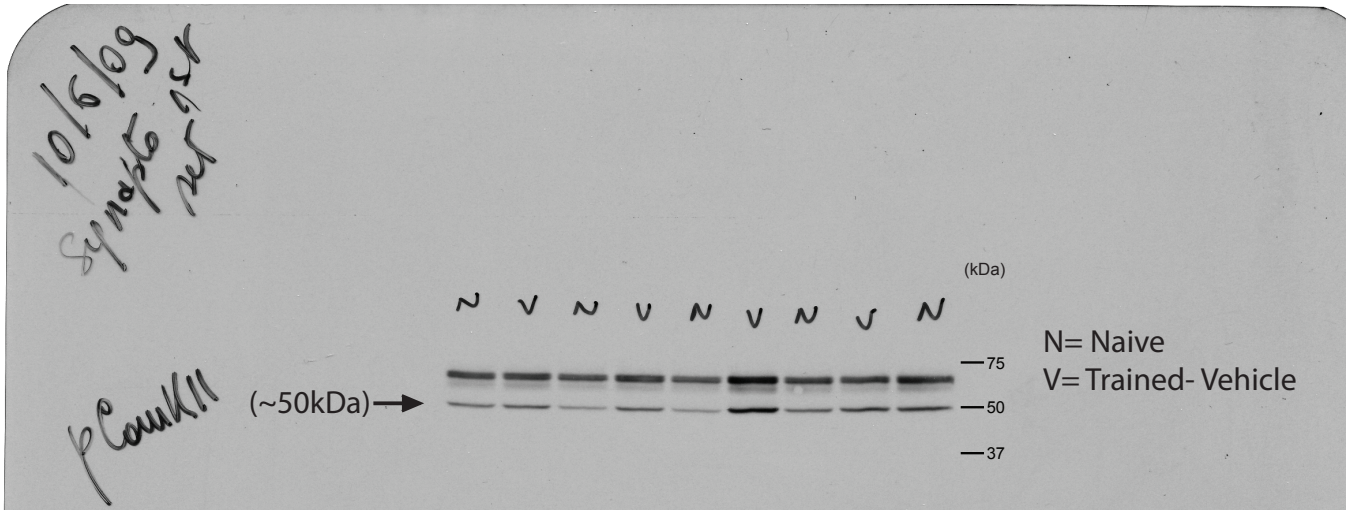
ACTIN



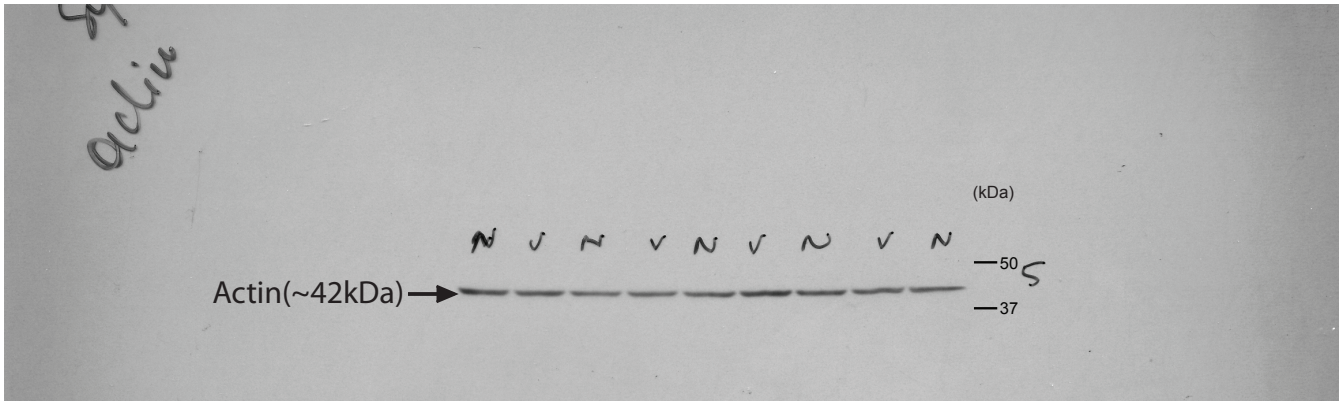
Supplementary Figure 15. Examples of full–length western blots of the Arc data shown in Fig. 4a. Red arrows indicate the samples that were cropped for the representative images of the Arc for the Trained-Vehicle vs Anti–BDNF group shown in Fig. 4a (Arc) and the corresponding actin bands (same membrane reprobed).

Chen et al. Supplementary Fig. 15

pCaMKIIα Fig 2a.



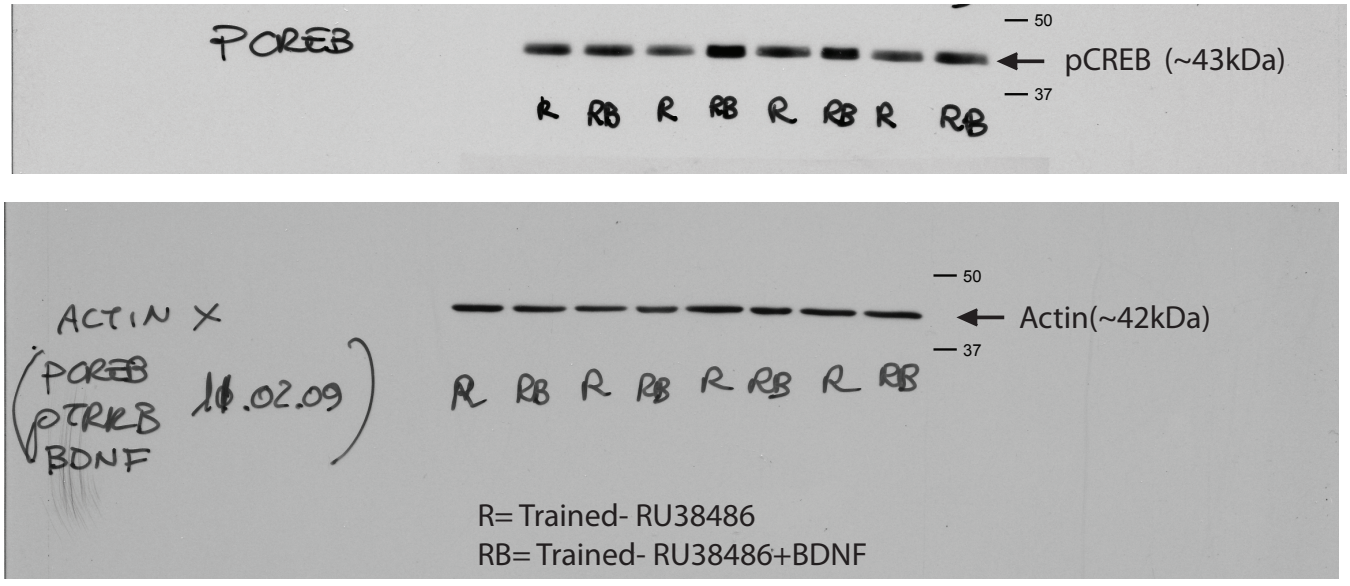
ACTIN



Supplementary Figure 16: **Full–length western blot examples for the quantitative pCamKIIα results shown in Fig. 2a.** The same membrane was first probed with an anti-pCamkIIα antibody, stripped and then reprobed with an anti-actin antibody.

Chen et al. Supplementary Fig. 16

pCREB Fig 6b.



Supplementary Figure 17: Full–length western blot examples for the quantitative pCREB results shown in Fig. 6b. The same membrane was first probed with an anti-pCREB antibody, stripped and then reprobed with an anti-actin antibody.

Chen et al. Supplementary Fig. 17

Supplementary Tables

Supplementary Table 1: Mean latencies ± s.e.m. of rats after training and treatment related to Figure 1.

Figure 1a	**Mean Latency (s)**			
	Acq.	Test 1 (T1)	Test 2 (T2)	Test 3 (T3)
Veh (n=6 rats)	19.38±1.1	291.5±43.8	313.1±80.5	320.2±69.7
RU486 (n=6 rats)	10.1±1.8	66.8±19.6	49.1±18.6	51.5±22.7
Statistics	Two–way ANOVA followed by Bonferroni post hoc tests Treatment: $F_{1,20} = 24.07$, $P < 0.0001$ Time: $F_{1,20} = 0.06$, $P = 0.80$ Time x Treatment: $F_{1,20} = 0.38$, $P = 0.54$			Student's *t*–test $P = 0.004$
Figure 1b	**Mean Latency (s)**			
	Acq.	Test 1 (T1)	Test 2 (T2)	Test 3 (T3)
Veh (n=6 rats)	14.9±5.1	326.8±64.6	252.3±49.4	274.9±50.2
RU486 (n=6 rats)	13.4±3.7	120.7±59.0	63.8±27.1	105.3±53.2
Statistics	Two–way ANOVA Treatment: $F_{1,20} = 12.85$, $P = 0.0019$ Time: $F_{1,20} = 0.98$, $P = 0.33$ Time x Treatment: $F_{1,20} = 0.017$, $P = 0.90$			Student's *t*–test $P = 0.043$
Figure 1c	**Mean Latency (s)**			
	Acq.	Test 1(T1)	Test 2 (T2)	Test 3 (T3)
Veh (n=6 rats)	12.8±2.8	322±60.6	307.5±68.5	342.9±94.2
RU486 (n=6 rats)	17.9±4.9	73.6±18.9	71.6±38.9	95.6±57.7
Statistics	Two–way ANOVA Treatment: $F_{1,20} = 22.92$, $P < 0.0001$ Time: $F_{1,20} = 0.03$, $P = 0.87$ Time x Treatment: $F_{1,20} = 0.015$, $P = 0.90$			Student's *t*–test $P = 0.049$
Figure 1d	**Mean Latency (s)**			
	Acq.	Test 1(T1)	Test 2 (T2)	
Veh (n=7 rats)	15.38 ± 4.3	435.6 ± 29.6	386.4 ± 39.7	
RU486 (n=6 rats)	12.18 ± 5.3	322.7 ± 65.8	249.2 ± 99.1	
Statistics	Two–way ANOVA Treatment: $F_{1,22} = 4.20$, $P = 0.053$ Time: $F_{1,22} = 1.02$, $P = 0.32$ Time x Treatment: $F_{1,20} = 0.038$, $P = 0.85$			
Figure 1e	**Mean Latency (s)**			
	Acq.	Test 1 (T1)		
Veh (n=5 rats)	11.4 ± 3.4	233.7 ± 32.9		
RU486 (n=5 rats)	10.7 ± 2.9	305.8 ± 68.9		
Statistics		Student's *t*–test $P = 0.37$		

Acq.: Acquisition latency.

Supplementary Table 2: Percentage fold change ± s.e.m. relative to naïve rats (a,b) or trained rats injected with vehicle (c) from western blot analyses and one–way ANOVA *F* values and Student's *t*–test *P* values related to Figure 2 and Supplementary Figure 2.

Figure 2a	**Fraction**	**Naïve**	**Veh**	**RU486**	**ANOVA *F* value**
pCREB	Total	100.0±14.4% (n=9 rats)	220.6±37.6% (n=8 rats)	149.5±16.9% (n=9 rats)	F (2,25) = 6.23, P = 0.0069
CREB	Total	100.0±17.5% (n=6 rats)	107.6±24.7% (n=6 rats)	111.1±6.0% (n=6 rats)	F (2,17) = 0.1014, P = 0.9041
pTrkB	Total	100.0±9.5% (n=9 rats)	105.5±16.3% (n=9 rats)	56.8±13.1% (n=9 rats)	F(2,26) = 3.564, P = 0.044
TrkB	Total	100±15.5% (n=5 rats)	105.9±14.6% (n=5 rats)	106.7± 13.9% (n=5 rats)	F (2,14) = 0.0621, P = 0.9401
Arc	Total	100.0±19.8% (n=8 rats)	470.0±79.3% (n=8 rats)	263.2±56.4% (n=7 rats)	F(2,22) = 10.89, P = 0.0006
pMSK1	Total	100±12.3% (n=11 rats)	99.1±14.2% (n=8 rats)	77.2±12.7% (n=9 rats)	F (2,27) = 0.97, P = 0.3928
MSK1	Total	100±6.3% (n=11 rats)	103.1±11.2 (n=8 rats)	94.2±9.1% (n=9 rats)	F (2,27) = 0.256, P = 0.7761
pCaMKIIα	SN	100.0±6.7% (n=9 rats)	245.9±37.5% (n=8 rats)	70.9±21.8% (n=9 rats)	F(2,25) = 14.48, P < 0.0001
CaMKIIα	SN	100.0±6.08% (n=10 rats)	109.4±7.2% (n=8 rats)	98.5±10.3% (n=10 rats)	F (2,27) = 0.4717 P =0.6294
GluA1	SN	100.0±5.0% (n=8 rats)	205.4±34.4% (n=8 rats)	135.9±18.6% (n=9 rats)	F(2,24) = 5.464, P = 0.0118
pSynapsin–1	SN	100.0±11.6% (n=5 rats)	116.7±4.0% (n=5 rats)	106.4±18.3% (n=5 rats)	F(2,14) = 0.439, P = 0.655
pERK1	SN	100.0±8.9% (n=8 rats)	96.5±15.5% (n=8 rats)	57.8±4.8% (n=8 rats)	F(2,23) = 4.813, P = 0.019
ERK1	SN	100.0±16.1% (n=8 rats)	71.2±10.8% (n=8 rats)	88.0±12.3% (n=9 rats)	F (2,24) = 1.154, P = 0.3337
pERK2	SN	100.0±11.5% (n=10 rats)	75.2±5.9% (n=8 rats)	48.9±6.4% (n=10 rats)	F(2,27) = 9.34, P = 0.0009
ERK2	SN	100.0±10.9% (n=8 rats)	86.6±12.4% (n=9 rats)	127.8±19.3% (n=8 rats)	F (2,24) = 2.105, P = 0.1457
pAkt	SN	100.0±20.4% (n=5 rats)	94.5±6.1% (n=6 rats)	59.3±5.8% (n=6 rats)	F(2,16) = 3.75, P = 0.049
Akt	SN	100.0±14.7% (n=8 rats)	104.64±12% (n=8 rats)	116.8±13.4% (n=8 rats)	F(2,23) = 0.5323, P = 0.595
pPLC–γ	SN	100.0±10.8% (n=8 rats)	85.4±11.8% (n=7 rats)	43.9±5.3% (n=8 rats)	F(2,22) = 9.63, P = 0.0012
PLC–γ	SN	100.0±13.4% (n=10 rats)	73.8±8.8% (n=7 rats)	82.1±6.2% (n=11 rats)	F (2,27) = 1.669, P = 0.209
Figure 2b	**Fraction**	**Naïve**	**Veh**	**RU486**	**ANOVA *F* value**
pCREB	Total	100.0±17.2% (n=8 rats)	153.2±12.9% (n=8 rats)	101.9±11.7% (n=8 rats)	F(2,23) = 4.57, P = 0.0225
CREB	Total	100.0 ± 5.3% (n=6 rats)	103.1± 10.6% (n=5 rats)	99.2 ± 11.8% (n=6 rats)	F(2,16) = 0.044, P = 0.9575
pTrkB	Total	100.0±12.05% (n=8 rats)	81.3 ± 6.3% (n=8 rats)	72.1 ± 5.3% (n=7 rats)	F(2,22) = 2.666, P = 0.0941
TrkB	Total	100.0±8.1% (n=6 rats)	88.0±20.5% (n=5 rats)	97.3±24.6% (n=5 rats)	F(2,15) = 0.1211, P = 0.8869

pCaMKIIα	SN	100.0±11.2% (n=8 rats)	187.4±25.3% (n=7 rats)	83.2±13.9% (n=7 rats)	$F(2,21) = 8.05$, $P = 0.0029$
CaMKIIα	SN	100.0±8.5% (n=8 rats)	93.8±10.9% (n=8 rats)	100.4±10.4% (n=8 rats)	$F(2,23) = 0.1373$, $P = 0.8725$
pSynapsin–1	SN	100.0±22.1% (n=8 rats)	217.8±30.1% (n=7 rats)	77.3±8.3% (n=7 rats)	$F(2,21) = 11.2$, $P = 0.0006$
Synapsin–1	SN	100.0±15.8% (n=7 rats)	106.9 ±21.4% (n=8 rats)	86.6 ± 8.4% (n=8 rats)	$F(2,22) = 0.4345$, $P = 0.6535$
pERK1	SN	100.0±8.0% (n=7 rats)	113.1±10.8% (n=8 rats)	107.7±18.6% (n=7 rats)	$F(2,21) = 00229$, $P = 0.793$
ERK1	SN	100.0±9.1% (n=8 rats)	122.0±11.4% (n=7 rats)	117.4±11.2 (n=8 rats)	$F(2,22) = 1.211$, $P = 0.3190$
pERK2	SN	100.0±4.8% (n=7 rats)	104.7±7.6% (n=8 rats)	102.6±13.9% (n=7 rats)	$F(2,21) = 0.0636$, $P = 0.9385$
ERK2	SN	100.0±8.6% (n=7 rats)	103.6±9.6% (n=8 rats)	113.5±7.3% (n=7 rats)	$F(2,21) = 0.6225$, $P = 0.5472$
pAkt	SN	100.0±11.4% (n=7 rats)	97.9±14.2% (n=8 rats)	111.8±18.5% (n=7 rats)	$F(2,21) = 0.2482$, $P = 0.7827$
Akt	SN	100.0±17.1% (n=7 rats)	110.7±11.7% (n=8 rats)	119.0 ± 7.4% (n=7 rats)	$F(2,21) = 0.5427$, $P = 0.5899$
GluA1	SN	100.0±8.75% (n=11 rats)	106.5±12.1% (n=8 rats)	97.3±8.0% (n=6 rats)	$F(2,24) = 0.1973$ $P = 0.8224$
Figure 2c	**Fraction**	**Veh**	**ActinoD**		**Student's *t*–test *P* value**
Arc	Total	100.0±10.56% (n=6 rats)	67.48±5.62% (n=5 rats)		$P = 0.0309$
pTrkB	Total	100.0±9.37% (n=6 rats)	113.1±19.6% (n=6 rats)		$P = 0.5590$
Zif268	Total	100.0±10.85% (n=8 rats)	60.8±14.09% (n=8 rats)		$P = 0.0445$
pCREB	Total	100.0±18.07% (n=6 rats)	111±12.03% (n=6 rats)		$P = 0.6230$
pCamKII	SN	100.0±9.15% (n=6 rats)	81.4±18.2% (n=5 rats)		$P = 0.3607$
GluA1	SN	100.0±8.5% (n=6 rats)	103.7±13.7 % (n=6 rats)		$P = 0.8214$

Total = Total cell lysate, SN = Synaptoneurosomal lysate; ActinoD = Actinomycin D

Supplementary Table 3: Mean latencies ± s.e.m. of rats after training and treatment related to Figure 3.

Figure 3a	**Mean Latency (s)**			
	Acq.	Test 1 (T1)	Test 2 (T2)	Test 3 (T3)
IgG (n=14 rats)	12.5±1.6	221.5±51.7	224.3±53.9	307.2±69.2
Anti–BDNF (n=18 rats)	12.6±8.7	50.7±10.1	43.9±12.5	70.9±33.6
TrkB–Fc (n=13 rats)	8.9±4.7	84.7±39.2	61.9±23.1	45.8±13.4
Statistcs	Two–way ANOVA Treatment: $F_{2,84} = 15.66$, $P < 0.0001$ Time: $F_{1,84} = 0.105$, $P = 0.747$ Time x Treatment: $F_{2,84} = 0.073$, $P = 0.929$			One–way ANOVA $F_{2,44} = 9.842$, $P = 0.003$
Figure 3b	**Mean Latency (s)**			
	Test 1 (T1)			
IgG (n=9 rats)	126.1 ± 36.4			
Anti–BDNF (n=9 rats)	98.3 ± 30.9			
TrkB–Fc (n=9 rats)	120.7 ± 50.6			
Statistics	One–way ANOVA $F_{2,26} = 0.1348$, $P = 0.8745$			
Figure 3c	**Mean Latency (s)**			
	Acq.	Test 1(T1)	Test 2 (T2)	Test 3 (T3)
IgG (n=6 rats)	9.8±3.3	303.2±65.8	299.0±77.3	370.4±77.5
TrkB–Fc (n=7 rats)	14.5±3.9	78.6±32.1	62.4±10.0	83.7±23.0
Statistics	Two–way ANOVA Treatment: $F_{1,22} = 21.21$, $P < 0.0001$ Time: $F_{1,22} = 0.041$, $P = 0.8413$ Time x Treatment: $F_{1,22} = 0.0143$, $P = 0.9058$			Student's *t*–test $P = 0.003$

Acq.=Acquisition latency.

Supplementary Table 4: Percentage fold change ± s.e.m. of naïve rats from western blot analyses and one–way ANOVA *F* values related to Figure 4 and Supplementary Figure 7.

Figure 4a	**Fraction**	**Naïve**	**IgG**	**Anti–BDNF**	**ANOVA *F* Value**
pCREB	Total	100.0±10.5% (n=5 rats)	290.5±33.4% (n=5 rats)	186.4±23.1% (n=5 rats)	F (2,14) = 15.61, P = 0.0005
CREB	Total	100.0±11.5% (n=6 rats)	105.8±20.7% (n=5 rats)	121.5±7.59% (n=6 rats)	F (2,16) = 0.725, P = 0.5017
Arc	Total	100.0±20.6% (n=7 rats)	318.9±48.1% (n=7 rats)	318.1±54.2% (n=6 rats)	F (2,19) = 9.093, P = 0.0021
pCaMKIIα	SN	100.0±6.7% (n=7 rats)	304.3 ± 54.6% (n=8 rats)	320.3 ± 70.1% (n=8 rats)	F (2,22) = 4.975, P = 0.0176
CaMKIIα	SN	100.0±7.2% (n=6 rats)	116.1±8.5% (n=6 rats)	111.9±18.5% (n=8 rats)	F (2,19) = 0.315, P = 0.7338
GluA1	SN	100.0±8.2% (n=5 rats)	223.4±43.3% (n=5 rats)	247.8±38.0% (n=8 rats)	F (2,17) = 4.444, P = 0.0305
pERK1	SN	100.0±9.9% (n=9 rats)	120.0±20.4% (n=9 rats)	61.7±5.9% (n=9 rats)	F (2,25) = 4.28, P = 0.0275
ERK1	SN	100.0±16.3% (n=9 rats)	86.7±12.4% (n=9 rats)	121.7±20.6% (n=8 rats)	F (2,25) = 1.122, P = 0.3428
pERK2	SN	100.0±8.9% (n=10 rats)	86.3±10.9% (n=8 rats)	57.9±8.2% (n=8 rats)	F (2,25) = 5.22, P = 0.0135
ERK2	SN	100.0±11.0% (n=9 rats)	75.3±10.2% (n=8 rats)	115.4±22.0% (n=8 rats)	F (2,24) = 1.434, P = 0.2598
pAkt	SN	100.0±7.9% (n=5 rats)	108.7 ± 19.9% (n=6 rats)	70.4±6.1% (n=6 rats)	F (2,16) = 2.352, P = 0.1316
Akt	SN	100.0±6.5% (n=5 rats)	90.9±15% (n=6 rats)	94.3±17.1% (n=6 rats)	F (2,16) = 0.098, P = 0.9077
pPLC–γ	SN	100.0±8.0% (n=5 rats)	86.6±7.2% (n=5 rats)	50.6±10.8% (n=5 rats)	F (2,14) = 8.422, P = 0.0052
PLC–γ	SN	100.0±15.0% (n=5 rats)	86.0±5.6% (n=5 rats)	82.6±11.1% (n=5 rats)	F (2,14) = 2.126, P = 0.1157
Figure 4b	**Fraction**	**Naïve**	**IgG**	**Anti–BDNF**	**ANOVA *F* Value**
pCREB	Total	100.0±16.5% (n=8 rats)	161.2±14.9% (n=8 rats)	88.2±8.6% (n=8 rats)	F (2,23) = 7.809, P = 0.0029
CREB	Total	100.0 ± 5.5% (n=8 rats)	104.2 ± 9.36% (n=8 rats)	120.7±11.0% (n=7 rats)	F (2,22) = 1.502, P = 0.2466
pCaMKIIα	SN	100.0±18.5% (n=8 rats)	167.7±29.3% (n=8 rats)	48.2±10.8 (n=10 rats)	F (2,25) = 10.17, P = 0.0007
CaMKIIα	SN	100.0±14.5% (n=6 rats)	84.3±14.3% (n=5 rats)	110.3±10.2% (n=5 rats)	F (2,15) = 0.895, P = 0.4325
pSynapsin–1	SN	100.0±25.5% (n=7 rats)	241.8±21.6% (n=6 rats)	85.0±16.1% (n=8 rats)	F (2,20) = 15.67, P = 0.0001
Synapsin–1	SN	100.0±17.9% (n=6 rats)	106.5±24.7% (n=7 rats)	116.3±12.9% (n=8 rats)	F (2,20) = 0.196, P = 0.8234
pERK1	SN	100.0±4.5% (n=6 rats)	117.4±16.1% (n=5 rats)	95.0±17.3% (n=6 rats)	F (2,16) = 0.698, P = 0.1157
ERK1	SN	100.0±9.1% (n=6 rats)	120.0±13.8% (n=6 rats)	118.7±14.5 (n=6 rats)	F (2,17) = 0.855, P = 0.4449
pERK2	SN	100.0±3.4% (n=6 rats)	106.6±9.4% (n=6 rats)	90.0±12.1% (n=6 rats)	F (2,17) = 0.8512 P = 0.4465
ERK2	SN	100.0±10.2%	103.6±12.1%	110.4±8.8%	F (2,17) = 0.255,

		(n=6 rats)	(n=6 rats)	(n=6 rats)	$P = 0.7781$
pAkt	SN	100.0±10.6% (n=7 rats)	107.5±12.0% (n=6 rats)	98.4±13.2% (n=7 rats)	$F(2,19) = 0.156$, $P = 0.8572$
Akt	SN	100.0±18.4% (n=7 rats)	112.7±12.7% (n=6 rats)	101.2±11.7% (n=7 rats)	$F(2,19) = 0.203$, $P = 0.8183$

Total = Total cell lysate, SN = Synaptoneurosomal lysate

Supplementary Table 5: Mean latencies ± s.e.m. of rats after training and treatment related to Figure 5.

Figure 5a	**Mean Latency (s)**		
	Acq.	Test 1 (T1)	Test 2 (T2)
Veh&PBS (n=8 rats)	10.3±1.9	261.3±37.6	243.7±48.3
RU486&PBS (n=8 rats)	14.1±4.5	81.7±19.5	61.9±23.1
RU486&BDNF (n=8 rats)	21.3±4.7	310.0±74.0	294.1±65.8
Statistics	Two–way ANOVA Treatment: $F_{2,44} = 13.44, P < 0.0001$ Time: $F_{1,44} = 0.2185, P = 0.6425$ Time x Treatment: $F_{2,44} = 0.001, P = 0.999$		
Figure 5b	**Mean Latency (s)**		
	Test 1 (T1)	Test 2 (T2)	
Veh/PBS (n=12 rats)	301.1 ± 42.4	302.2 ± 29.9	
Veh/BDNF (n=12 rats)	227.1 ± 20.5	206.6 ± 31.2	
RU486/PBS (n=11 rats)	97.0 ± 36.9	65.8 ± 22.2	
RU486/BDNF (n=10 rats)	242.7 ± 36.7	221.7 ± 72.3	
RU486/NGF (n=9 rats)	134.9 ± 49.7	65.9 ± 28.5	
RU486/NT–3 (n=8 rats)	122.0 ± 37.6	110.9 ± 62.0	
Statistics	Two–way ANOVA Treatment: $F_{5,112} = 9.414, P < 0.0001$ Time: $F_{1,112} = 1.163, P = 0.2832$ Time x Treatment: $F_{5,112} = 0.1749, P = 0.9715$		
Figure 5c	**Mean Latency (s)**		
	Acq.	Test 1(T1)	Test 2 (T2)
Veh&PBS (n=6 rats)	16.6 ± 3.4	368.3 ± 67.1	299.0±77.3
Prop&PBS (n=7 rats)	14.0 ± 2.3	59.7 ± 24.9	92.3±57.9
Prop&BDNF (n=8 rats)	16.2 ± 3.9	148.2 ± 62.1	78.0 ± 30.8
Statistics	Two–way ANOVA Treatment: $F_{2,36} = 17.44, P < 0.0001$ Time: $F_{1,36} = 0.3516, P = 0.5569$ Time x Treatment: $F_{2,36} = 0.5369, P = 0.5892$		

Acq.=Acquisition latency.

Supplementary Table 6: Percentage fold change ± s.e.m. of trained rats injected with vehicle from western blot analyses and one–way ANOVA *F* values related to Figure 6.

Figure 6a	**Fraction**	**Veh**	**RU486**	**RU486+BDNF**	**ANOVA *F* Value**
pCREB	Total	100.0 ± 13.0% (n=8 rats)	60.8 ± 7.7% (n=8 rats)	99.2 ± 6.7% (n=8 rats)	F (2,23) = 5.543, P = 0.0117
CREB	Total	100.0 ± 22.9% (n=5 rats)	125.5±28.7% (n=6 rats)	95.9 ± 21.9% (n=5 rats)	F (2,15) = 0.4185, P = 0.6666
pTrkB	Total	100.0 ± 15.5% (n=7 rats)	53.9 ± 12.4% (n=7 rats)	113.4 ± 14.0% (n=8 rats)	F (2,21) = 4.916, P = 0.019
TrkB	Total	100.0 ± 13.8% (n=5 rats)	100.7 ± 13.8% (n=5 rats)	92.0 ± 6.6% (n=5 rats)	F (2,14) = 0.1727, P = 0.019
Arc	Total	100.0 ± 10.0% (n=8 rats)	56.3 ± 12.0% (n=7 rats)	45.1 ± 11.0% (n=5 rats)	F (2,19) = 7.006, P = 0.0060
pCaMKIIα	SN	100.0 ± 15.3% (n=9 rats)	28.9 ± 8.9% (n=9 rats)	50.4 ± 8.3% (n=8 rats)	F (2,25) = 10.44, P = 0.0006
CaMKIIα	SN	100.0 ± 7.34% (n=6 rats)	98.7 ± 8.6% (n=7 rats)	98.3 ± 9.5% (n=6 rats)	F (2,18) = 0.0103, P = 0.9898
GluA1	SN	100.0 ± 12.7% (n=9 rats)	54.2 ± 7.2% (n=9 rats)	95.9 ± 20.2% (n=8 rats)	F (2,25) = 3.455, P = 0.0488
pERK1	SN	100.0 ± 17.0% (n=8 rats)	57.1 ± 8.4% (n=10 rats)	95.2 ± 17.3% (n=12 rats)	F (2,29) = 8411, P = 0.1148
ERK1	SN	100.0 ± 15.2% (n=10 rats)	123.6 ± 17.3% (n=9 rats)	119.6 ± 7.9% (n=10 rats)	F (2,28) = 0.8411, P = 0.4426
pERK2	SN	100.0 ± 14.2% (n=8 rats)	50.4 ± 5.1% (n=10 rats)	93.9 ± 15.4% (n=12 rats)	F (2,29) = 4.267, P = 0.0245
ERK2	SN	100.0 ± 14.3% (n=10 rats)	147.6 ± 22.2% (n=10 rats)	153.0 ± 8.9% (n=10 rats)	F (2,29) = 3.286, P = 0.0528
pAkt	SN	100.0 ± 8.5% (n=5 rats)	67.0 ± 9.3% (n=5 rats)	87.5 ± 6.8% (n=5 rats)	F (2,14) = 4.064, P = 0.0449
Akt	SN	100.0 ± 11.5% (n=8 rats)	111.7 ± 12.8% (n=8 rats)	115.2 ± 6.8% (n=6 rats)	F (2,21) = 0.491, P = 0.6195
pPLC–γ	SN	100.0 ± 13.8% (n=7 rats)	51.4 ± 6.4% (n=11 rats)	125.6 ± 12.3% (n=9 rats)	F (2,26) = 14.63, P < 0.0001
PLC–γ	SN	100.0 ± 11.9% (n=5 rats)	91.1 ± 6.1% (n=5 rats)	93.8 ± 10.1% (n=5 rats)	F (2,14) = 0.2224, P = 0.8038
Figure 6b	**Fraction**	**Veh**	**RU486**	**RU486+BDNF**	**ANOVA *F* Value**
pCREB	Total	100.0 ± 8.4% (n=8 rats)	66.6 ± 7.7% (n=8 rats)	90.0 ± 10.8% (n=8 rats)	F (2,23) = 3.606, P = 0.0451
CREB	Total	100.0 ± 10.3% (n=5 rats)	96.2 ± 9.9% (n=6 rats)	112.0 ± 11.5% (n=5 rats)	F (2,15) = 0.6074, P = 0.5595
pCaMKIIα	SN	100.0 ± 14.2% (n=7 rats)	44.4 ± 7.4% (n=8 rats)	94.1 ± 16.2% (n=8 rats)	F (2,22) = 5.522, P = 0.0123
CaMKIIα	SN	100.0 ± 11.7% (n=8 rats)	107.1 ± 11.1% (n=8 rats)	125.1 ± 18.0% (n=8 rats)	F (2,13) = 0.8598, P = 0.4376
pSynapsin–1	SN	100.0 ± 13.8% (n=7 rats)	35.5 ± 5.8% (n=7 rats)	54.3 ± 9.5% (n=7 rats)	F (2,20) = 12.81, P = 0.0003
Synapsin–1	SN	100.0 ± 20.0% (n=7 rats)	81.0 ± 7.8% (n=8 rats)	62.8 ± 4.9% (n=8 rats)	F (2,22) = 2.399, P = 0.1165

Total = Total cell lysate, SN = Synaptoneurosomal lysate